## **SUPPLEMENTARY MATERIAL**

for

# Multisensory Flicker Modulates Widespread Brain Networks and Reduces Interictal Epileptiform Discharges in Humans

## **Authors**

Lou T. Blanpain<sup>1,2,3</sup>, Emily Chen<sup>1</sup>, James Park<sup>1</sup>, Michael Y. Walelign<sup>4</sup>, Robert E. Gross<sup>1</sup>, Brian T. Cabaniss<sup>5</sup>, Jon T. Willie<sup>6\*</sup>, Annabelle C. Singer<sup>2,3\*</sup>
\*co-corresponding authors

## **SUPPLEMENTARY TABLES:**

Subject	Sex	Age	Language dominance (as determined by fMRI)
01	F	26-30	L
02	M	31-35	L
03	F	46-50	L
04	F	26-30	L
05	F	46-50	L
06	F	21-25	L
07	M	21-25	L
08	M	51-55	В
09	M	36-40	L
10	M	26-30	L
11	F	21-25	R
12	F	31-35	L
13	F	26-30	L
14	F	21-25	L
15	M	31-35	L
16	F	21-25	L

Table S1. Subject demographics

M - male; F - female; R - right; L - left; B - bilateral.

Subject	Prescribed AEDs	AEDs on day of testing	Preoperative imaging findings	Determined seizure focus
01	Zonisamide, lamotrigine, levetiracetam	Lamotrigine, levetiracetam, zonisamide	History of left hippocampal sclerosis, expected post-operative findings of mesial temporal ablation.	Left temporal
02	Lamotrigine, levetiracetam, topiramate	Lamotrigine, levetiracetam, topiramate	History of prior left medial occipital-parietal resection, possible bilateral hippocampal sclerosis.	Right temporo- occipital region
03	Eslicarbazepine, lamotrigine	Lamotrigine	Likely left hypothalamic hamartoma.	Bilateral medial temporal
04	levetiracetam, lamotrigine, lorazepam, gabapentin	Flicker 5.5-40-80Hz session: lamotrigine, levetiracetam Single-pulse session: none	Question of medial left temporal cortical displasia.	Left medial temporal
05	Clobazam, levetiracetam, phenytoin.	Flicker 5.5-40-80Hz session: none Single-pulse session: levetiracetam, lorazepam	Right hemispheric atrophy, right mesial temporal sclerosis.	Right temporo- occipital region
06	Lamotrigine, lacosamide, perampanel.	Flicker 5.5-40-80Hz session: lamotrigine Single-pulse session: lamotrigine	Expected post-operative findings of left medial temporal ablation.	Left posterior parahippocampal area
07	Topiramate, phenytoin, gabapentin, clonazepam	Flicker 5.5-40-80Hz session: phenytoin Single-pulse session: none	Left frontal lobe polymicrogyria and associated closed-lip scattered schizencephaly.	Left fronto- parietal region
08	Lacosamide, lamotrigine	Lacosamide, lamotrigine	Expected post-operative findings of left temporal pole ablation.	Left orbitofrontal region
09	Lamotrigine	Flicker 5.5-40-80Hz session: lamotrigine Single-pulse session: none	Possible anterior right frontal focal cortical dysplasia.	Left mesial temporal
10	Levetiracetam, zonisamide, Lamotrigine.	Flicker 5.5-40-80Hz session: lamotrigine Single-pulse session: lamotrigine, levetiracetam	Possible right hippocampal atrophy.	Bilateral medial temporal
11	Lacosamide	None	Bilateral occipital periventricular nodular heterotopia.	Right parieto- occipital region

12	Levetiracetam, lamotrigine	Flicker 5.5Hz-80Hz range session: none Flicker 5.5-40-80Hz session 1: lamotrigine, levetiracetam Flicker 5.5-40-80Hz session 2: none	No abnormal findings	Left medial temporal
13	Levetiracetam, zonisamide	None	No abnormal findings	Left basal/lateral temporal
14	Clobazam, lamotrigine, perampanel	Clobazam, lamotrigine	Small left frontal white matter cavernous malformation with associated developmental venous anomaly.	Left posterior frontal (perirolandic)
15	Carbamazepine, levetiracetam	None	History of radiosurgery of left temporal lobe and frontal operculum arteriovenous malformation.	Left planum temporale, Heschl's gyrus, pars opercularis.
16	Levetiracetam, lamotrigine	Flicker 5.5-80Hz range session 1: lamotrigine (100mg). Flicker 5.5-80Hz range session 2: levetiracetam (250mg).	History of left temporal lobe low-grade (WHO grade 1) tumor, bilateral gray matter heterotopias, hypothalamic hamartoma, retrocerebellar cyst.	Poorly localized, multifocal; onset possibly left mesial temporal, bilateral, or multifocal

<u>Table S2. Epilepsy information for each subject</u> AED – anti-epileptic medication.

Subject	IED rate (IED/min)	Seizure events			
		clinical	subclinical	total	
01	15.7	22	6	28	
02	122.7	1	+	1+	
03	79.9	13	4	17	
04	32.6	5	0	5	
05	61.0	11	20	31	
06	30.0	5	1	6	
07	9.6	5	0	5	
08	54.5	4	0	4	
09	36.6	7	0	7	
10	44.5	4	+	4+	
11	n/a	11	0	11	
12	47.4	2	0	2	
13	25.4	1	0	1	
14	n/a	17	1	18	
15	n/a	2	1		
<b>16</b> n/a		21 1		22	

Table S3. Intracranial monitoring activity per subject

 $IED-interictal\ epileptiform\ discharge;\ n/a-not\ available;\ +-multiple,\ not\ counted.\ IED\ rate\ was\ based\ on\ number\ of\ IEDs\ detected\ over\ the\ duration\ of\ the\ Flicker\ 5.5-40-80Hz\ experimental\ session.$ 

	Paradigm					
Subject	Flicker 5.5-40-80Hz		Single-pulse		Flicker 5.5-80Hz range	
	Brightness (Lux)	Volume (dbA)	Brightness	Volume	Brightness	Volume
01	163	76	n/a	n/a	n/a	n/a
02	199	82	n/a	n/a	n/a	n/a
03	14	83	n/a	n/a	n/a	n/a
04	49	78	715	93	n/a	n/a
05	136	72	1029	96	n/a	n/a
06	815	72	1003	74	n/a	n/a
07	122	80	162	96	n/a	n/a
08	1125	80	n/a	n/a	n/a	n/a
09	978	89	970	93	n/a	n/a
10	880	84	1063	100	n/a	n/a
11	n/a	n/a	n/a	n/a	158	n/a
12	212	79	n/a	n/a	233	n/a
13	189	77	n/a	n/a	n/a	n/a

Table S4. Paradigm and sensory stimulation amplitudes per subject

n/a

n/a

n/a

14

15

16

Total

n/a

n/a

n/a

12

A total of 16 subjects completed one of more of three paradigms (see Figure 1B, 4B, and Methods for details). For each subject and paradigm, participation as well as brightness and volume (averaged between left and right sides of the glasses or earbuds) measured at 40Hz are indicated.

6

n/a

n/a

n/a

n/a

n/a

n/a

n/a

n/a

82

5

85

95

79

#### **SUPPLEMENTARY FIGURES:**

#### A Flicker modulation in **B** Electrode coverage by paradigm occluded condition Flicker 5.5-40-80Hz Single-pulse Flicker 5.5-80Hz range 6 subjects 12 subjects 5 subjects 1896 contacts 1025 contacts 821 contacts 6 (fold-change in power) Occluded condition 5 ...... 3 4 5 6 Stimulation condition (fold-change in power)

Figure S1. Relative occluded condition modulation and electrode coverage by paradigm

(A) Out of contacts that showed significant flicker modulation to 40Hz visual, auditory or audiovisual flicker in the Flicker 5.5-40-80Hz paradigm, we represented the corresponding fold-change in power (capped at 7) at the frequency of stimulation for the relative occluded condition versus the non-occluded condition. Each dot indicates a contact's responses for a given modality in one recording session with orange, green and blue dots representing visual (V), audio-visual (AV), or auditory (A) stimulus conditions, respectively, and dots circled in black representing results that are significant in the relative occluded condition. Significant modulation in the occluded condition may suggest our occluded condition is not completely successful in occluding sensory stimuli from the subject's visual and auditory systems. For rare cases where we observed a clear peak at the frequency of stimulation in the PSD for the occluded condition, in the majority of those cases the peak was smaller than in the non-occluded condition, which suggests it may be true sensory modulation from imperfect occlusion of the sensory stimuli, rather than noise from the flicker device. Overall, the vast majority of contacts show stronger modulation in the non-occluded condition. This indicates low noise levels using our experimental and preprocessing methods.

(B) Number of subjects and number of contacts, as well as approximate location of each contact (represented by dots) across patients on Montreal Neurological Institute (MNI) normalized 3D brain (top view) for each of the three paradigms tested (see Figure 1B, 4B, and 5A for details).

# A Hippocampus units

20µV

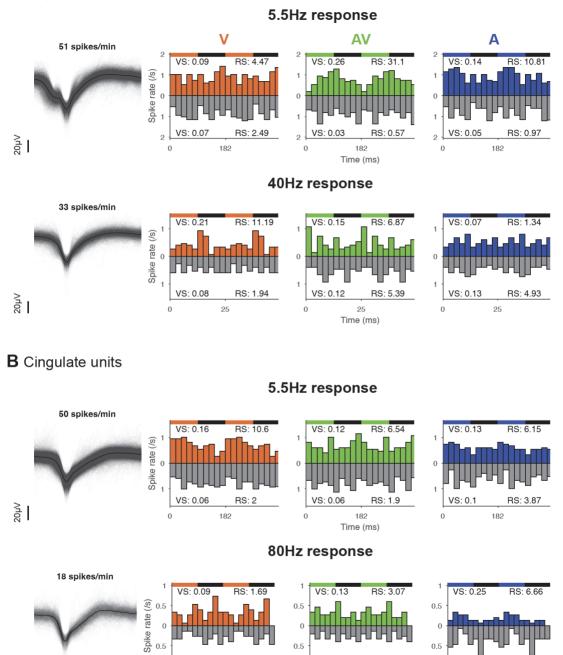


Figure S2. Flicker modulates neurons' spiking activity in the human hippocampus and cinqulate (A) Example single neuron waveforms (left; solid line represents average waveform, transparent lines represent individual waveforms), with peristimulus-time histograms (right) averaged over 2 cycles of the stimulus, illustrating from left to right response to 5.5Hz visual (V, orange), audiovisual (AV, green), and auditory (A, blue) stimulation (colored bars) versus random condition (grey inverted bars). Vector strength (VS) and Rayleigh statistics (RS) for each condition are indicated on the top and bottom of the plot. We see a higher average firing rate at a given phase of the

VS: 0.04

0

RS: 0.29

12.5

Time (ms)

RS: 2.71

12.5

VS: 0.12

0

RS: 3.93

12.5

VS: 0.13

stimulus for the AV condition, showing that this unit is more strongly modulated by 5.5Hz-AV flicker. Bottom: same illustration for a hippocampal multi-unit, in response to 40Hz flicker. This unit seems to be more strongly modulated in the visual modality.

(B) Same as (A) for cingulate units, showing response to 5.5Hz flicker (top) and 80Hz flicker (bottom). Top shows single neuron with stronger modulation to 5.5Hz-V stimulation, while bottom shows multi-unit with stronger modulation to 80Hz-A stimulation.

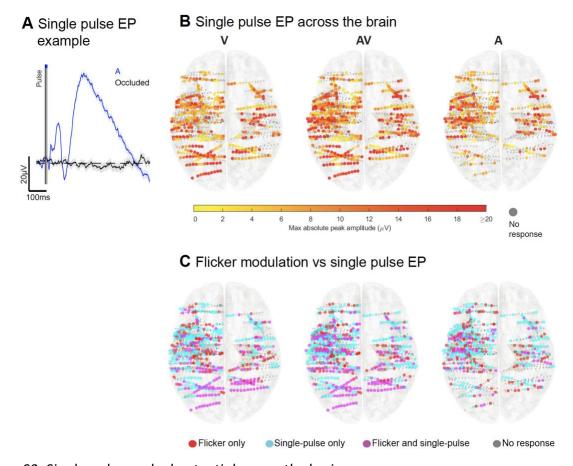


Figure S3. Single-pulse evoked potential across the brain

(A) Example evoked potential (EP), averaged across 200 trials, to auditory (A, blue) versus relative occluded audio-visual (black) pulses, in the primary auditory cortex; solid line represents the mean, shaded area represents standard error of the mean. As expected, we see a rapid (first peak  $^{\sim}20$ ms), large (up to  $^{\sim}50\mu$ V) response to auditory pulse compared to the relative occluded condition.

(B) Approximate location and associated single pulse EP amplitudes of contacts (illustrated with dots) represented on 3D Montreal Neurological Institute (MNI) normalized brain (top view), for visual (V, left), audio-visual (AV, center) and auditory (A, right) modalities, capped at  $20\mu V$ .

Smaller grey dots represent non-significant single pulse EP responses, while large dots represent significant responses, with maximal absolute peak from  $0\mu V$  (yellow) to  $20\mu V$  or more (red). There were 97, 148 and 71 contacts with amplitude values higher than  $20\mu V$  respectively in the visual, audio-visual, and auditory modalities. As expected, we see a strong response to conditions involving the visual modality in the occipital region, but also in the parietal, temporal, and prefrontal regions. Strong responses to the auditory condition were observed in the temporal region, but also the prefrontal region.

(C) Responses to single-pulse versus flicker: approximate location of contacts (represented by dots) and their responses to visual (left), audiovisual (middle) and auditory (right) modalities, represented on 3D MNI brain (top view). Contacts show responses to flicker-only (red), single pulse-only (cyan), both flicker and single pulses (purple), or no response (grey).

## A Visual modulation

# **B** Auditory modulation

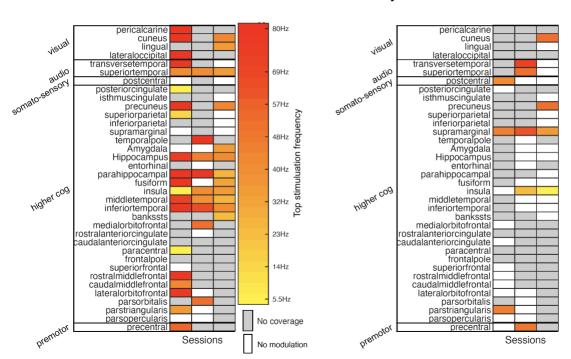


Figure S4. Preferred stimulation frequency by brain region and modality

(A) Representation of the stimulation frequency leading to maximal fold-change in power in each respective brain region, in the case of visual stimulation. Only contacts showing significant fold-change in power to more than six of the stimulation frequencies tested, were included in the analysis. Moreover, when multiple of such channels were located to a given region, the channel responding to the highest number of frequencies, was picked in order to determine top stimulation frequency for that region.

(B) Same as (A) but for sessions involving auditory stimulation.